Supplementary information

Materials and methods

Chemicals and solvents

Tetraethoxysilane (TEOS), 3-(trimethoxysilyl)propyl methacrylate (TMPM), polyvinylpyrrolidone MW = 10,000 g/mol (PVP) and NH_3 (25%) were purchased from Sigma-Aldrich. NIPAM was purchased from Polysciences, Inc.; AA (99.5%) from Alfa Aesar; MBA (99.5%, electrophoretic grade) and sodium dodecylsulfate (SDS, GC grade) from Sigma; and AIBN (98%) from Aldrich. *N*-Propargyl methacrylamide (AlkMA) was synthesized by acryloylation of propargylamine.[1]

Reagents were purified as follows: AIBN was recrystallized once from ethanol without heating, NIPAM was dissolved in acetone and recrystallized twice from hexanes, AA was distilled under vacuum, and MBA was recrystallized once from acetone.

DMSO (99.9%) was purchased from Sigma-Aldrich, ethanol (HPLC grade) from VWR, and methanol (HPLC grade) from Fisher Chemicals. Water was deionized with a Milli-Q purification system.

The following were used for preparation of Britton-Robinson buffers: NaOH (p.a., Lach-Ner), acetic acid (99.5%, Alfa Aesar), H_3PO_4 (p.a., Penta), H_3BO_3 (99.8% ACS reagent, Sigma-Aldrich), and KCl (p.a., Penta).

Nuclear magnetic resonance

^1H NMR spectra were acquired on a Bruker Avance IIIHD 600 MHz spectrometer (600.1 MHz for ^1H) in D_2O (referenced to 1,4-dioxane as an external standard, $\delta(1H) = 3.75$ ppm).

Infrared (IR) spectroscopy

IR spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} on a Nicolet 6700 spectrometer equipped with an Agilent 6850 gas chromatograph using KBr pellets. The sample weight was 1.0 mg/pellet for all samples.

Thermogravimetry

Thermogravimetry was measured with a TG-750 instrument (Stanton Redcroft, England). Approximately 1 mg powder sample was heated under air with a heating rate of 20 °C/min.

Dynamic light scattering (DLS), zeta potential (ZP) and conductivity measurements

Particle diameters and ZPs in 15 mM Britton-Robinson buffers of constant conductivity and various pHs were recorded with a Zetasizer Nano ZPS system (Malvern Instruments) at physiologically relevant temperatures. Sample concentrations were 0.2 mg/ml. As all samples showed a single peak, the particle diameter was measured as the z-average diameter evaluated by the instrument software. ZP was measured concurrently with sample conductivity with a ZEN1002 dip cell. ZP and DLS measurements were repeated three times for each pH value, and errors were calculated as standard deviations from the mean. Average conductivity and conductivity error were calculated from all 18 obtained values (i.e., 3 replicates for 6 samples).

Fluorescence measurements

Fluorescence spectra were measured using a home-built spectrometer (Figure S1)[2]. This device is specialized for measuring fluorescence spectra of highly scattering and low-fluorescent samples in solution. Spectra are collected under a 180° measurement angle and read by an ultra-high sensitivity PMA12, C10027-02 detector equipped with a BT-CCD image sensor (Hamamatsu). A DPSS CW 514.4 nm laser, 100 mW (Cobolt Fanfango 04-01 Series), serves as an excitation source. Emission and excitation beams are separated by a long-pass 532 nm dichroic mirror (Chroma). For measurement at 37 °C, a Peltier heating plate (TECI 12714, Vigan) added to the cuvette holder is controlled by software developed in-house. The temperature for each sample was equilibrated for 15 min.

For all measurements the sample concentration was 0.2 mg/mL. Spectra were collected in each measurement 300 times with an exposure time of 1 s. The intensities of NV^0 ZPL and spectral maxima were evaluated as follows: intensities for each measurement were smoothed by elimination of outliers and averaged, and the background intensity measured under the same conditions was subtracted. The resulting values were used for max/NV^0 ratio calculation. The errors were calculated as standard deviations from three measurements.

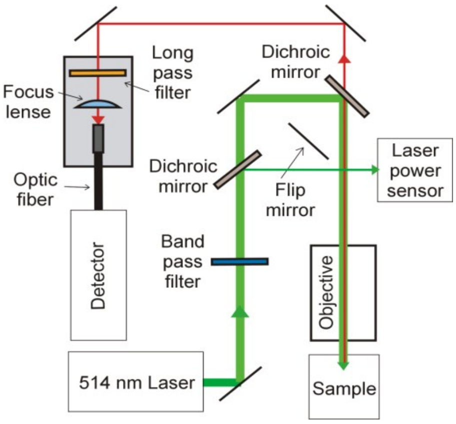

Figure S1: Scheme of the home-built fluorescence spectrometer.

Synthetic procedures

FND pretreatment and irradiation

NDs were supplied by Microdiamant Switzerland (MSY 0-0.05). The particles contained approximately 100-200 ppm of natural nitrogen impurities. NDs were oxidized by air in a Thermolyne 21100 tube furnace at 510 °C for 5 h and subsequently treated with a mixture of HNO_3 and H_2SO_4 (85 °C, 3 days), washed with 0.1 M NaOH and 0.1 M HCl, washed five times with water, and freeze-dried. Purified ND powder (1552 mg) was pressed in an aluminum target holder and irradiated with a 16.6 MeV electron beam extracted from an MT25 microtron for 21 h (1.25×10^{19} cm^{-2} fluence). The irradiated material was annealed at 900 °C for 1 h under an argon atmosphere and subsequently oxidized for 6 h at 510 °C in air. The NDs were then treated with a mixture of HNO_3 and H_2SO_4 (85 °C, 3 days), washed with 0.1 M NaOH and 0.1 M HCl, washed five times with water, and freeze-dried. Prior to use, the particles were dissolved in water (2 mg/mL) and sonicated with a probe (Cole-Parmer, 750 W) for 30 min. The resulting transparent colloid was filtered using a 0.45 µm GMF filter.

FND@sil preparation: Coating of FNDs with a methacrylate-terminated thin silica layer

Polyvinylpyrrolidone (68 mg, 6.8 µmol) was dissolved in water (60 mL) and sonicated for 10 min in an ultrasonic bath. Filtered FND colloid (12 mL, 2 mg/mL; i.e., 24 mg FNDs) was added, and the mixture was stirred for 24 h. The colloid was then concentrated via centrifugation in two steps. In the first step (40,000 rcf, 1 h), the volume was reduced to approximately 6 mL. The second centrifugation step (30,000 rcf, 30 min) was performed in microvials and reduced the solvent volume to approximately 1.5 mL. Sedimented NDs were resuspended in ethanol (24 mL) in a round bottom flask and sonicated in an ultrasonic bath for 2 min. TEOS (168 mg, 805 µmol) and 3-(trimethoxysilyl) propylmethacrylate (62.7 mg, 250 µmol) were added. After 2 min of vigorous stirring, ammonia solution (25%, 1 mL) was added, and the reaction mixture was stirred for 14 h. The product was purified by two-step centrifugation with HPLC-grade methanol (25 mL, 4x): first at 15,000 x g for 15 min, then the supernatant was collected and centrifuged at 25,000 x g for 20 min. The concentrated methacrylated silica-coated particles were stored in the freezer (–18 °C) as a stable colloid or used directly for polymerization.

FND@RS preparation: Synthesis of the responsive polymeric shell

A dispersion of 24 mg silica-coated NDs in ethanol was transferred in several portions into 600 µL of DMSO in a rotary evaporator. In another vial, the following reagents were dissolved in 1.8 mL DMSO: freshly recrystallized NIPAM (136 mg), MBA (14.64 mg), freshly distilled AA (8.28 mg), AlkMA (8.04 mg), and SDS (4.56 mg). Freshly recrystallized AIBN (60 mg) was added. This mixture was transferred into the FND@sil solution, sealed, and secured by 3 cycles of vacuum/argon. The reaction proceeded for 3 days at 55 °C with moderate stirring under an argon atmosphere. After polymerization, the reaction mixture was divided into 4 microvials, diluted twice with HPLC-grade ethanol, and centrifuged (15 min at 15,000 x g, then the supernatant was collected and centrifuged again for 20 min at 40,000 x g). The pellets were redispersed, merged, and washed with ethanol four additional times under similar centrifugation conditions as the first time. The concentration of the washed colloidal solution in ethanol was determined gravimetrically, and particles were stored in the freezer at –18 °C. We note the importance of monomer purity. Synthesis of some batches of FND@RS was not successful in our hands, presumably because of an insufficient care about the starting monomers (see above).

Optimization of polymerization conditions

Optimization of polymerization reaction mixtures was performed in the same manner as described above with 2 mg samples. The total concentration of monomers, their ratio, and the AIBN concentration were adjusted. The total monomer concentration was in the range of 0.3 mol/L to 0.61 mol/L. Higher concentrations led to compact gel formation. The molar ratio of NIPAM:AA was kept at 1:10 according to Pinheiro et al.,[3] and the molar ratio of MBA:(NIPAM+AA) was changed from 1:20 to 1:5 for various total monomer concentrations. AIBN concentration was reduced from 0.3 mol/L to 0.15 mol/L due to gel formation.

Miscellaneous

Composition of Britton-Robinson buffers

Following the work of Mongay and Cerda,[4] buffers of desired pH values were prepared, and their total ionic concentration was adjusted with KCl to 0.15 mol/L. The actual measured pH values did not precisely match the published values, but were slightly shifted (see Table S1). Slight differences in conductivity were adjusted by addition of small amounts of KCl. For all particle measurements, buffers were diluted 10 times to obtain concentration of 0.015 mol/L and conductivity of 1.79 ± 0.09 mS cm^{-1} at 25°C. As conductivity is a function of temperature, its value for measurements at 37 °C was 2.16 ± 0.13 mS cm^{-1}, and thus constant conductivity conditions were kept only for constant temperature measurements. These values were obtained concurrently with ZP measurements. In some cases, such as for reversibility of pH responses, the solution conductivity was measured with a COND7 conductometer probe (XS Instruments).

pH	NaOH (g)	CH_3COOH (mL)	H_3PO_4 (mL)	H_3BO_3 (g)	KCl (g)
4.65	0.0800	0.0925	0.1095	0.0990	0.4145
4.97	0.0981	0.0873	0.1033	0.0934	0.3821
5.68	0.1091	0.0841	0.0995	0.0900	0.3582
6.18	0.1143	0.0826	0.0977	0.0884	0.3463
7.02	0.1288	0.0784	0.0928	0.0839	0.3090
7.87	0.1461	0.0734	0.0869	0.0785	0.2304

Table S1. Composition of Britton-Robinson buffers for total volume 50 mL and total concentration 0.15 mol/L.

Reversibility measurements

To measure the reversible behavior of particles, we started with 2.5 mL of sample (0.2 mg/mL) in a buffer with a pH of 7.1 and a conductivity of 1.79 S cm^{-1}. Subsequently, the pH was adjusted to 5.0 by addition of several microliters of 1 M HCl or to 7.1 by addition of 1 M NaOH. Then, the solution conductivity was adjusted by addition of several tens or hundreds microliters of water, and particle diameter was measured by DLS.

Additional figures

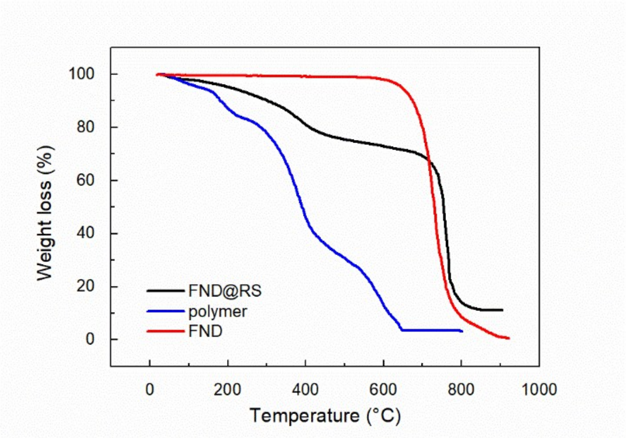

Figure S2: Thermogravimetric analysis of FND@RS, pure responsive polymer, and naked FNDs.

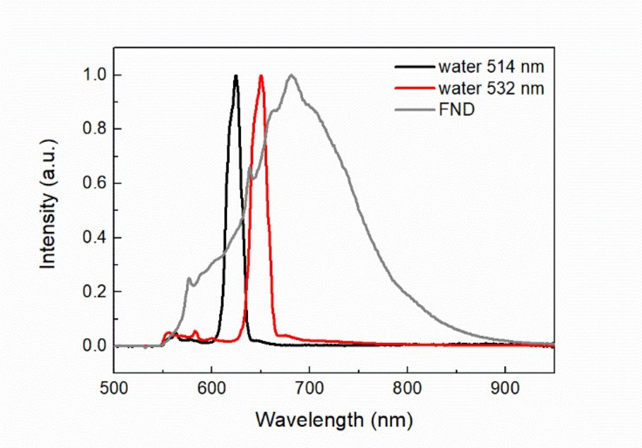

Figure S3: Comparison of water Raman peaks for two excitation laser wavelengths (514 and 532 nm) with FND fluorescence spectra. The spectra are normalized to their maxima. While the Raman peak of water excited at 532 nm (which is commonly used for excitation of NV$^-$) coincide with NV$^-$ ZPL, the 514 nm laser shows much lower spectral interference. Nevertheless, even for 514 nm excitation we chose the spectral maximum instead of NV$^-$ ZPL because of its higher intensity and spectral distance from the Raman peak of water (624 nm).

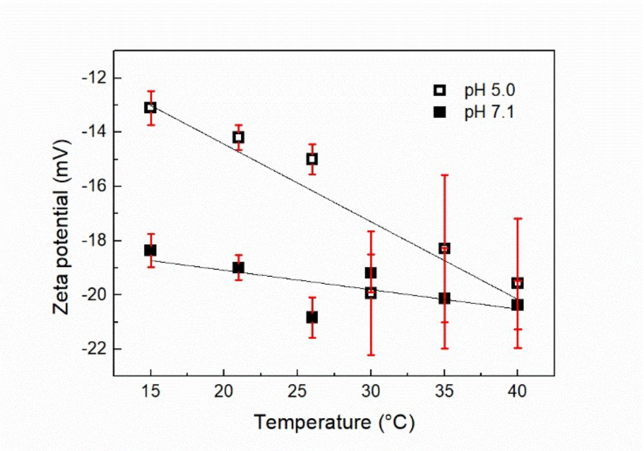

Figure S4: Temperature dependence of zeta potential of FND@RS for pH 5.0 and 7.1.

References

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